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Biotechnology of southern African bulbs

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The greatest diversity of bulbs, including petaloid monocotyledons with corms, rhizomes and tubers, can be found in the Cape Floral Kingdom in southern Africa. Biotechnological approaches are used to meet the growing demand for bulbs and to conserve those over-exploited by the herbal medicine trade. This review outlines the principles and practices of micropropagation, flower bulb improvement, *in vitro* conservation, molecular marker applications and secondary metabolite production for southern African bulbs and discusses the future role of bulbs and biotechnology in the development of the region.

Introduction

Southern Africa, with a rich flora comprising an estimated 30 000 species, has been singled out as the bulb capital of the world since it is home to more bulbs than any other region. Although known from both the winter and summer rainfall areas in South Africa, the greatest diversity of bulbs belongs to the Cape Floral Kingdom. They make a spectacular show when they appear after a dormant season and, perhaps, this is why many have become part of the traditions and folklore of southern Africa. Certainly they were much prized by the explorers who came to the Cape of Good Hope. Early records of plant hunting expeditions recall their discovery and shipment to Europe in order to meet the growing demand for novelty by the horticultural trade (Bryan 1989, McCracken and McCracken 1990). Today, the carpets of blooms in spring provide a dazzling display for visitors to the Cape.

These geophytes, or bulbs, as they are often called, are a group of plants that produce underground storage organs, including corms, rhizomes, tubers and bulbs (Du Plessis and Duncan 1989). Most are petaloid monocotyledons with showy flowers like gladioli, freesia, calla lilies and chinkerinchees. They are well known to European gardeners and much in demand as cut flowers, pot plants and as garden ornamentals. Current horticultural practices include biotechnological tools — a 'body of techniques that use biological systems, living organisms, or derivatives thereof to make or modify products or processes for specific use' (Convention on Biological Diversity, Article 2, United Nations Environment Programme, 1992).

This review outlines the principles and practices of micropropagation, flower bulb improvement, *in vitro* conservation, molecular marker applications and secondary metabolite production and addresses future research needs in the biotechnology of southern African bulbs.

Bulbs and Biotechnology

Several African countries have established research programmes in the biotechnology of ornamental crops and geophytes, including Burundi, Kenya, South Africa and Zimbabwe (Brink *et al.* 1998). In South Africa, research and development at the Agricultural Research Council (ARC), National Botanical Institute (NBI), tertiary education institutions and businesses in the private domain, focuses on the tissue culture and long term storage of ornamental bulbs, genetic engineering and the use of molecular markers for identifying new cultivars (Brink *et al.* 1998).

Micropropagation

A wide variety of bulbs from southern Africa, both ornamental and medicinal, have been propagated *in vitro* (Table 1) (Krikorian and Kann 1986, George 1993, Kim and De Hertogh 1997, McCartan and Van Staden 1999) although those grown for cut flower production, such as *Freesia*, *Gladiolus* and *Nerine*, have received most attention. Bulblet production involves several key stages: explant selection and decontamination; the initiation of growth; multiplication of the propagules; storage organ formation; and transfer *ex vitro*. For a more detailed account of these stages, the reader is referred to earlier reviews by George (George 1993) and Kim (Kim and De Hertogh 1997).

Explants: selection and decontamination
Although explants from many different tissues have an inherent capacity to regenerate plantlets, meristematic structures will not form on all explants with the same frequency (Van Aartrijk and Van der Linde 1986, Boonekamp 1997). For bulbous plants, scales from the basal regions where they are joined to the basal plate; slices of young,

Table 1: Families of southern African bulbs (excluding the Orchidaceae) propagated through tissue culture

Family	Genus	References
Dicotyledons		
Oxalidaceae	*Oxalis* spp.	Kim and De Hertogh 1997
Monocotyledons		
Alliaceae	*Agapanthus* sp.	Hussey 1980, Krikorian and Kann 1986
	Tulbaghia sp.	Zschocke and Van Staden 2000
Amaryllidaceae	*Amaryllis belladonna*	De Bruyn *et al.* 1992
	Clivia miniata	Krikorian and Kann 1986, LiChao *et al.* 1995, Kim and De Hertogh 1997
	Crinum spp.	Krikorian and Kann 1986, Slabbert *et al.* 1993, 1995, Fennell *et al.* 2001
	Cyrtanthus spp.	Kukulczanka and Kromer 1988, McAlister *et al.* 1998b, Niederwieser and Kleynhans 1998
	Gethyllis linearis	Drewes and Van Staden 1994
	Haemanthus spp.	Rabe and Van Staden 1999
	Nerine spp. and cultivars	Krikorian and Kann 1986, Mochtak 1989, Lilien-Kipnis *et al.* 1990, Custers and Bergervoet 1992, Lilien-Kipnis *et al.* 1992, George 1993, Lilien-Kipnis *et al.* 1994, Ziv *et al.* 1995, Kim and De Hertogh 1997, Vishnevetsky *et al.* 1997, Ziv and Lilien-Kipnis 1997a, Vishnevetsky *et al.* 2000
Araceae	*Zantedeschia* sp.	Kritzinger *et al.* 1998, Kubo *et al.* 2002
Asphodelaceae	*Kniphofia pauciflora*	McAlister and Van Staden 1996
Colchicaceae	*Gloriosa superba*	Finnie and Van Staden 1989, Samarajeewa *et al.* 1993, Custers and Bergervoet 1994, Sivakumar and Krishnamurthy 2000, Takamura *et al.* 2002
	Sandersonia aurantiaca	Finnie and Van Staden 1989
Hyacinthaceae	*Bowiea volubilis*	George 1993, Hannweg *et al.* 1996
	Drimia robusta	Ngugi *et al.* 1998
	Eucomis spp.	De Lange *et al.* 1989, Ault 1995a, McCartan and Van Staden 1995, McCartan *et al.* 1999, Taylor and Van Staden 2001a
	Galtonia spp.	Drewes and Van Staden 1993, Kim and De Hertogh 1997
	Lachenalia spp. and cultivars	Van Rensburg and Vcelar 1989, George 1993, Ault 1995b, Kim and De Hertogh 1997, Slabbert and Niederwieser 1999
	Ornithogalum spp. and cultivars	Krikorian and Kann 1986, Van Rensburg *et al.* 1989, Landby and Neiderwieser 1992, George 1993, Wangai and Bock 1996, Kim and De Hertogh 1997, Ziv and Lilien-Kipnis 1997a, Ziv and Lilien-Kipnis 1997b
	Schizobasis intricata	Drewes *et al.* 1993
	Scilla spp.	Josekutty *et al.* 1998, McCartan and Van Staden 1998, Crouch *et al.* 1999, McCartan and Van Staden 1999, McCartan and Van Staden 2002
	Thuranthos basuticum	Jones *et al.* 1992
	Velthemia spp. and cultivar	Ault 1996, Taylor and Van Staden 1997
Hypoxidaceae	*Hypoxis* spp.	Appleton and Van Staden 1995a, Appleton and Van Staden 1995b
	Rhodohypoxis baurii	Upfold *et al.* 1992
Iridaceae	*Babiana* spp.	Jäger *et al.* 1995, McAlister *et al.* 1998a
	Crocosmia sp.	Krikorian and Kann 1986
	Dierama latifolium	Page and Van Staden 1985
	Freesia spp. and cultivars	Krikorian and Kann 1986, Bach 1992, George 1993, Wang *et al.* 1994, Kim and De Hertogh 1997
	Gladiolus spp. and cultivars	Krikorian and Kann 1986, George 1993, Dantu and Bhojwani 1995, Remotti 1995, Remotti and Loffler 1995, Nagaraju *et al.* 1996, Kim and De Hertogh 1997, Jäger *et al.* 1998, Pathania *et al.* 2001, Kumar *et al.* 2002
	Ixia spp.	Sutter 1986, Dinkelman and Van Staden 1988, Meyer and Van Staden 1988
	Schizostylis sp.	Krikorian and Kann 1986, George 1993
	Sparaxis sp.	Krikorian and Kann 1986, Kim and De Hertogh 1997

unelongated flower stems and buds that have already differentiated, have proved to be the most successful explants, although the choice may depend on the family to which the selected bulb belongs. For while bulbs, leaves, inflorescence stems and ovaries of the Liliaceae all produce plantlets, only bulbs and inflorescence stems are productive in the Iridaceae and Amaryllidaceae (Hussey 1978).

There are advantages to using inflorescence stems rather than explants from the bulb since floral tissues, at anthesis, are: (1) at a stage where the plant breeder can assess the plant for desirable floral traits; (2) equipped with a large number of potential meristems; (3) relatively pathogen-free and (4) able to be excised from the plant without destroying the bulb (Ziv and Lilien-Kipnis 1997a, 2000). However, not all bulbs flower every year and there is the possibility that off-types may be produced (Pierik and Steegmans 1986). Young peduncles which are known to be more successful in regenerating plantlets than mature floral stems (Pierik 1991, Jacobs *et al.* 1992, Lilien-Kipnis *et al.* 1992, Ziv *et al.* 1995) must also be excised before they emerge from the bulb.

Bulb, twin- or tri-scales are used as explants from mature bulbs since they are known to freely produce shoots *in vitro*. The regeneration potential may, however, be species and genotype dependent, with the number of buds per isolated explant ranging from 2–25 (Ziv and Lilien-Kipnis 2000). In the Amaryllidaceae, for example, no bulblets form in the absence of the basal plate (Pierik and Ippel 1977, George 1993). Tri-scales and mini-chips are used especially when the addition of plant growth regulators has no stimulatory effect on the smaller twin-scales (Squires and Langton 1990, Ulrich *et al.* 1999).

Since contamination is the most important reason for losses incurred in micropropagation systems, particularly among cultures initiated from soil-borne organs like bulbs and rhizomes, explants must first be decontaminated using a variety of available sterilants (George 1993, Leifert and Cassells 2001). Disinfection protocols for bulbs may include fungicide and hot water pretreatments to remove endogenous pathogens (Hol and Van der Linde 1992, George 1993, Gilad and Borochov 1993, Langens-Gerrits *et al.* 1998). They are especially useful when the contamination frequency of the outer, older parts of the bulb is high (Squires and Langton 1990).

The production of propagules

Success at stage one depends on the establishment of an aseptic culture followed by the growth of the explant. The propagules produced are either adventitious or axillary shoots, somatic embryos or meristematic clusters derived from liquid culture systems. Although embryogenic propagules have the potential for high-volume multiplication and are used for propagating some of even the most recalcitrant species (Bach 1992, Lilien-Kipnis *et al.* 1994, Wang *et al.* 1994, Ziv *et al.* 1994, Remotti and Loffler 1995), shoots are more frequently reported as the propagule produced at this stage. Shoot regeneration almost always requires a nutrient medium containing hormonal supplements (Van Aartrijk and Van der Linde 1986); the most critical being auxins and cytokinins. The requirement for exogenous plant growth regulators may depend on the endogenous levels, which, in turn are influenced by the duration and temperature of storage of the parent bulb. This means that for each bulb, the levels of growth-promoting substances must be empirically determined. Generally low concentrations of auxin ($<10^{-6}$M) increase the induction of cell divisions leading to shoot production in bulbous crops (Van Aartrijk and Van der Linde 1986). The promotive effect of cytokinins on shoot production has been reported for many species of monocotyledons where apical dominance normally inhibits shooting (Hussey 1976). Compared to the Iridaceae, amaryllidaceous species require higher cytokinin levels to promote branching (Hussey 1976). However, most monocotyledons require auxins and cytokinins in combination for maximum shoot production (Hussey 1978); although when bulbs are used as explant sources, cytokinins may not be essential (Van Aartrijk and Van der Linde 1986).

Multiplication of the propagules

Multiplication is most often via one of two pathways; either division of shoot clumps (Chow *et al.* 1992, Bergoñón *et al.* 1996, Selles *et al.* 1997) or bulblets derived from shoots (George 1993). Rarely is callus used. Bulblets are cut lengthwise into halves or trimmed to remove the upper half to a third of the bulb. Adding benzyladenine (BA) to the medium improves the development of secondary shoots (Custers and Bergervoet 1992).

Liquid culture systems are growing in popularity for the mass propagation of important ornamentals (Bergoñón *et al.* 1992). This is because traditional methods of plant micropropagation are still largely labour intensive (Preil 1991), which makes them costly to use for crops other than those of high value (Ziv *et al.* 1995, Ziv 1997). In addition, growth and multiplication of bulbous plants in liquid systems occurs more rapidly, with an increase in the number of buds produced and better bulblet growth (Simmonds and Werry 1987, Ziv 1990, Takayama *et al.* 1991, Bergoñón *et al.* 1992, Takahashi *et al.* 1992a, 1992b, Chow *et al.* 1993, Niimi *et al.* 1997, Ziv 1997, Ziv and Lilien-Kipnis 1997b). Scaling-up in larger volumes, as in tank cultures, has enabled bulblets to be produced on an industrial scale e.g. *Gladiolus* and *Nerine* (Ziv 1997).

Storage organ formation

Storage organ formation, either from shoots or meristemoid clusters, occurs on solid media and is influenced by sucrose concentration, the balance of plant growth regulators, charcoal, temperature, and, in some cases, the photoperiod and ammonium/nitrate ratio (Peck and Cumming 1986, Ilan *et al.* 1995, Marinangeli and Curvetto 1997). Where bulblets develop directly from twin-scale explants, explant position, size and polarity may affect bulblet development (Pierik and Ippel 1977, Takayama and Misawa 1980, Huang *et al.* 1990). The advantages of inducing the formation of storage organs are that it reduces the need for rooting and acclimatisation (Ilan *et al.* 1995) and allows for long term storage (Ziv 1997).

Transfer to the natural environment

The final stage involves planting out the propagules, which, in the case of micropropagation schemes involving bulbous

plants, are bulblets (Chow *et al.* 1992). Rooting may facilitate plantlet establishment *ex vitro* and is stimulated by auxins (Drewes and Van Staden 1994) and activated charcoal (Ziv 1979). Survival may also be dependent on the size and mass of the bulblets (Hannweg *et al.* 1996) and, in some cases, cold treatment to break dormancy (Squires and Langton 1990). Conditions conducive to acclimatisation include a relatively high humidity and low light intensities (Kim and De Hertogh 1997, Ziv 1997). Bulblets generally transfer readily *ex vitro*, although the success rate may be dependent on the species or cultivar (Squires *et al.* 1991).

Flower bulb improvement

The production of disease-free plants

One of the benefits of propagating bulbs *in vitro* is the in-built disease protection it affords. This is of particular importance in producing disease-free plants for international trade. Viruses can be eliminated by meristem-tip culture, sometimes combined with thermo- and chemotherapy, and the disease-free condition maintained in successive clonal cultures (Kim and De Hertogh 1997, Ziv 1997). It is also possible to obtain virus-free plants by organ culture (using disease-free storage organs, young inflorescence stems and leaf tips) (Kim and De Hertogh 1997), and, in the case of *Freesia*, through the repeated subculture of callus (Kim and De Hertogh 1997). Plants can also be stored under disease-free conditions in suitable media at low temperatures to provide reserves of disease-free material for breeding lines (Bonnier and Van Tuyl 1997).

Plant breeding

In situ hybridisation is still widely used in the development of new cultivars in South Africa e.g. *Lachenalia* (Du Preez *et al.* 2002, Kleynhans *et al.* 2002) and in making available new cultivars of popular genera like *Gladiolu*s. *Veltheimia bracteata* 'Lemon Flame', *Zantedeschia aethiopica* 'Green Goddess' and *Lachenalia* and *Oxalis* cultivars are among several recent releases of bulbous ornamentals in South Africa (Jansen van Vuuren 1995) although there is still much scope for the selection and development of new horticultural varieties (Stirton 1980, Ferreira and Hancke 1985, Jansen van Vuuren *et al.* 1993, Jansen van Vuuren 1995, Niederwieser *et al.* 2002b).

In vitro techniques have important practical applications in plant breeding, particularly in speeding up the breeding process (Krikorian and Kann 1986). For flower bulbs the objective is to improve disease resistance as well as physiological traits such as ease of forcing and year-round flowering (Le Nard 2000). Although there are several *in vitro* techniques used to modify horticultural traits (Kim and De Hertogh 1997, Ziv 1997), *in vitro* hybridisation remains the most important technique for flower bulbs (Le Nard 2000). *In vitro* pollination, fertilisation and embryo rescue are useful in overcoming pre- and post-fertilisation barriers, especially for difficult and incompatible interspecific and intergeneric crosses (Debergh 1994, Ziv 1997) while haploid plants, from cultured anthers, pollen grains, ovaries or unfertilised ovules, are used to produce homozygous diploids by spontaneous or colchicine-induced doubling of the chromosome number e.g. *Gladiolus* and *Freesia* (Kim and De Hertogh 1997, Ziv 1997). Somaclonal variants, which occur in callus cultures of some geophytes following continuous subculture, can potentially be used to develop disease resistance (Kim and De Hertogh 1997, Ziv 1997). Although invaluable in the production of novel somatic hybrids, in overcoming incompatibility barriers and in introducing new characteristics (Fowler 1986, Dodds 1991), protoplast isolation has, to date, been limited to a few bulb species (Ziv 1997) and there are no reports for somatic hybrids among geophytes (Kim and De Hertogh 1997).

Transgenic flower bulbs

Genetic transformation is employed in the improvement of horticultural traits (Ziv 1997) especially where conventional techniques are unsuccessful and because novel hybrids can be produced in a shorter period of time. To date *Agrobacterium*-mediated transformation and biolistics (particle bombardment) have been used to genetically transform *Gladiolus* e.g. *Gladiolus* cv. Jenny Lee and *Ornithogalum* (Ziv 1997, Babu and Chawla 2000, De Villiers *et al.* 2000). In the case of *Ornithogalum* this was for the purpose of conferring resistance to the ornithogalum mosaic virus. Transgenic plants were produced by bombarding callus derived from leaf segments of a hybrid of *Ornithogalum thyrsoides* x *O. dubium* (De Villiers *et al.* 2000). *Agrobacterium* infection and transformation were achieved in cultivars of *Gladiolus* by prewounding shoot tips using a biolistic delivery system (Babu and Chawla 2000). Embryogenic cell lines of *Gladiolus* have also been transformed (Kamo *et al.* 2000). There are, however, still many problems to be overcome. Genes need to be identified, especially multigenes controlling floricultural characteristics (Ziv 1997).

In vitro *conservation*

Bulbs have not escaped the threats of habitat destruction and overexploitation as human settlements, agricultural practices and afforestation place increasing pressures on the environment and highly prized bulbs are harvested from the wild for the horticultural and medicinal plant trade (Read 1989, Newton and Bodasing 1994). In South Africa the problem is even more acute as the increasing demand for medicinal plants has meant that bulbs are harvested in an unsustainable way (Cunningham 1988, Mander 1998).

Collections of southern African bulbous and cormous plants, principally from the families Iridaceae and Liliaceae, are housed at The University of California (Irvine) (Koopowitz and Kaye 1983) and, in South Africa, there are gene banks, including *in vitro* material, for members of the Amaryllidaceae, Hyacinthaceae and Iridaceae at the ARC (Roodeplaat) (Jansen van Vuuren 1995). The conservation and maintenance of a viable bulb collection is, however, difficult for several reasons. These relate to the biology of the plants and, for members of the Amaryllidaceae, include long generation times, incompatibility factors and fleshy seeds that cannot be stored cryogenically in gene banks (Koopowitz and Kaye 1983, Koopowitz 1986). In other cases flowering may be erratic with the result that only a small percentage of the population produces viable seed. Even then,

seeds may be predated by insects.

In vitro techniques provide a means of conserving species that: have recalcitrant seeds; need to be vegetatively propagated; or are otherwise difficult to germinate. The majority of geophytic *Disa* species, particularly in the summer rainfall areas in South Africa, for example, exhibit consistently poor seed germination. Liquid cultures and cultures containing activated charcoal have, however, decreased the time taken for seeds to germinate and promoted the synchronicity of germination events for several species where previously this had not been possible (Thompson *et al.* 2001). For other geophytes, propagules are cryopreserved or stored under conditions that induce slow growth (Engelmann 1991). By reducing the nutrient strength and increasing the concentration of sucrose, vegetative material can be induced to undergo dormancy and thus be maintained for long periods (Bonnier and Van Tuyl 1997). These methods have yet to be used to advantage in the conservation of southern African bulbs.

Molecular marker applications

Molecular marker technology, although invaluable in the selection of genes for breeding, determining genetic diversity and in the identification of new cultivars, has not been used extensively for flower bulbs (Ziv 1997). Since it is useful to know to what extent variation among plants, either wild or hybrid, including somaclonal variants, exists, RAPD analyses are routinely employed in breeding programmes. In the case of *Lachenalia bulbifera*, for example, intraspecific variation was found to be high using RAPDs (Kleynhans and Spies 2000).

Secondary metabolite production

There are advantages to producing compounds in culture, especially where plants are endangered or slow growing. Year round production is also possible, and since plants are grown under standardised conditions, constant and stable supplies are guaranteed (Giulietti and Ertola 1999). Almost 50 different categories of compounds have so far been recovered from cell cultures (Bajaj *et al.* 1988) — mainly phenylpropanoids, alkaloids, terpenoids and quinones (Stöckigt *et al.* 1995). Since many bulbs are used in traditional medicine in southern Africa, there is growing interest in analysing their phytochemical constituents. For example, *Hypoxis* species contain rooperol, a compound with promising anti-cancer activity, while several *Eucomis* species exhibit anti-inflammatory activity. These results have naturally led workers to investigate the potential for producing secondary metabolites *in vitro*.

Hypoxoside, a phenolic glucoside found in the corms of several *Hypoxis* species, is used medicinally for the treatment of urogenital diseases (Bayley and Van Staden 1988). Root-type cultures of *Hypoxis rooperi* were initiated from flower buds and corms. Although production improved over time on media with reduced nitrogen and for those cultures that were grown in the dark, levels were considered too low for *in vitro* production to be viable (Page and Van Staden 1987).

Cultured *Eucomis* plantlets (*E. autumnalis* subsp. *autumnalis*) produce high levels of anti-inflammatory activity, much like they do in the wild. Callus initiated from leaf explants also showed anti-inflammatory activity, with greater inhibition of COX-2 inhibition (69%) than COX-1 inhibition (46%) (Taylor and Van Staden 2001c). A decrease in COX-1 inhibition occurred in plants grown on low concentrations of sucrose (10g l^{-1}) (Taylor and Van Staden 2001b).

Crinum alkaloids were isolated from the *in vitro* grown bulblets of *C. moorei*, including lycorine which has known pharmacological activity. Total alkaloid levels as well as those for specific alkaloids were influenced by the type of medium on which the bulblets were grown (Fennell *et al*. 2003).

Callus cultures of *Oxalis reclinata* produce a red pigment, cyanidin-3-glucoside, at 25°C in the light (Crouch *et al.* 1993), whereas in the dark or low light, the red pigment fails to accumulate (Makunga *et al.* 1997). Anthocyanin synthesis was promoted by increasing the concentration of the auxin 2,4-D from 0.5µM to 2µM and including BA, thidiazuron and zeatin, each at 8µM, and sucrose at 120–360µM, in the medium (Meyer and Van Staden 1995).

Excised root cultures of *Gloriosa superba* were found to accumulate approximately 240µg colchicine g^{-1} cell dry weight after four weeks' growth. The precursors p-coumaric acid + tyramine (each at 20mg l^{-1}) enhanced the colchicine content of the root cultures (Biswajit *et al.* 2002).

Biotechnology: A Tool for Development in Africa

Biotechnology plays a role in commercialising crops, creating new jobs and earning foreign exchange (Brink *et al.* 1998). This is of increasing importance in Africa where the population growth burdens environmental sustainability (Brink *et al.* 1998).

Economic development

Bulbs have long been an important commodity in trade, especially within the European Union (EU) and The Netherlands. In 1999, *Gerbera* and *Freesia* — both developed from genetic material from South Africa — were two of the top ten best sellers at the Dutch flower auctions, earning $143 million (Coetzee *et al.* 2002). Because South Africa's bulbs have, for centuries, been in the public domain, benefit sharing, in terms of profits, is 'fraught with difficulties' (Coetzee *et al.* 2002). South Africa has a wealth of flower bulbs, yet few have contributed to the commercial growing sector internationally (Niederwieser *et al.* 2002a), with the exception of *Gladiolus*, *Freesia*, calla (arums) and *Ornithogalum*. Even so, South Africa is an important supplier, earning 2.3 million Euros in exports in 2000 as the 7th highest ranked supplier to the EU (Eurostat). Coetzee (Coetzee *et al.* 2002) maintains that future benefits may be realised through cooperative agreements with international organisations or through the export of cut flowers and bulbs.

Bulbs also form an important source of income for local markets and rural communities in South Africa. Available by the 50kg bagful, bulbs form a large proportion of the material sold at medicinal plant markets (Cunningham 1988). In total, about 20 000 tonnes of plant material are traded on a

national level at medicinal markets with an estimated value of US$60 million or R270 million (Mander 1998).

The industry is beginning to stimulate new business opportunities. Cape Seed and Bulb, for example, recently initiated a small business specialising in the harvesting and cultivation of indigenous bulbs in Pella near Atlantis in the Cape. It's an important venture for rural communities which could earn them millions of rands (Jordan 1999). Nurseriwilde, an indigenous nursery specialising in bulbs, has also attracted attention after the owner, Robyn Mackenzie, was awarded the title of female farmer of the year in KwaZulu-Natal for 2002.

Social development

'The government is eager to assist local bulb growers to corner their rightful share of the world trade' (Jordan 1999). For this reason, there are moves to develop training centres for growing bulbs to international standards (Jordan 1999) with support from rural development companies. Projects like these encourage the sustainable production of natural resources although most have yet to take advantage of biotechnology strategies for improving plant production.

The cost of technology development and transfer

Even though biotechnology has the potential to increase the income of resource-poor farmers, technology development and transfer are costly. It is, therefore, important, not only to develop sustainable partnerships, but funding as well (Njobe-Mbuli 1999, Niederwieser *et al.* 2002a).

The Future

Biotechnology in Africa: Limitations and Prospects

Compared to developed countries, several factors limit the application of plant biotechnology in Africa: (1) inadequate infrastructure, (2) lack of skilled human resources and (3) availability of research equipment and facilities (Brink *et al.* 1998, Brink *et al.* 1999). South Africa launched an initiative in 2001 to stimulate growth in the biotechnology sector — a key theme of its national research and development strategy — to address these limitations (A National Biotechnology Strategy 2001). This is being achieved through the creation of a number of regional biotechnology initiatives. According to Brink, the development of biotechnology may best be accomplished in phases; firstly by introducing tissue culture, followed by tools to improve the selection and breeding of new plant varieties and, thirdly, by developing the capacity to produce transgenic plants (Brink *et al.* 1998).

Research Needs

Biotechnology is as important in developing countries as in the rest of the world, even though the needs may be quite different from those of industrial countries. In the case of flower bulbs, Le Nard and De Hertogh suggest that research needs to focus on the effects of external factors on physiological processes as well as the physiological and genetic control of bulb and flower induction (Le Nard and De Hertogh 2002). Attempts at sustainable bulb production, by reducing fertiliser and pesticide use, is already receiving attention in The Netherlands and may well lead to better cultivation practices worldwide (Jansma *et al.* 2002).

Conclusions

Southern Africa boasts a rich flora that includes more bulbs than any other region. Of all the biotechnology tools available, micropropagation is the most routinely used for conservation and to provide stocks of horticultural novelties. Protocols have been established for several genera but these have yet to be applied to a diversity of species. The application of *in vitro* techniques to flower bulb improvement, long term storage and secondary metabolite production has received comparatively little attention in the region. With the current focus on biotechnology and potential for selection and development of new horticultural varieties, biotechnology may well be regarded as a tool for development in Africa. Niederwieser cautions that this must be facilitated by sustainable funding, a multidisciplinary approach to research, market considerations, technology transfer and commercial growers (Niederwieser *et al.* 2002a).

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